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Determinants of Whether or Not Mixtures of Disinfection By-Products are Similar

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Reactive chemicals have been used to disinfect drinking waters for over a century. In the 1970s, it was first observed that the reaction of these chemicals with the natural organic matter (NOM) in source waters results in the production of variable, complex mixtures of disinfection by-products (DBP). Because limited toxicological and epidemiological data are available to assess potential human health risks from complex DBP mixture exposures, methods are needed to determine when health effects data on a specific DBP mixture may be used as a surrogate for evaluating another environmental DBP mixture of interest. Before risk assessors attempt such efforts, a set of criteria needs to be in place to determine whether two or more DBP mixtures are similar in composition and toxicological potential. This study broadly characterizes the chemical and toxicological measures that may be used to evaluate similarities among DBP mixtures. Variables are discussed that affect qualitative and quantitative shifts in the types of DBP that are formed, including disinfectants used, their reactions with NOM and with bromide/iodide, pH, temperature, time, and changes in the water distribution system. The known toxicological activities of DBP mixtures and important single DBPs are also presented in light of their potential for producing similar toxicity. While DBP exposures are associated with a number of health effects, this study focuses on (1) mutagenic activity of DBP mixtures, (2) DBP cancer epidemiology, and (3) toxicology studies to evaluate similarity among DBP mixtures. Data suggest that further chemical characterization of DBP mixtures and more systematic study of DBP toxicology will improve the quality and usefulness of similarity criteria.

The chemical disinfection of drinking water results in the formation of a large and complex array of disinfectant by-products (DBPs) that varies significantly with source water characteristics and water treatment scenarios. Because limited toxicological and epidemiological data are available to assess potential human health risks from complex DBP mixture exposures, parameters need to be identified that determine whether the risks posed by an environmental DBP mixture of concern differ from those associated with a DBP mixture that has been evaluated in a toxicological or epidemiological study. In addition, methods are needed for deciding whether the composition and toxicity of two or more DBP mixtures are sufficiently dissimilar such that the toxicity data for one is unlikely to adequately characterize the risks of the other (e.g., the magnitude or spectrum of adverse effects posed by exposure to the DBP mixture of concern).

The reactive chemicals used to disinfect drinking waters generate a myriad of DBPs upon reaction with the natural organic matter (NOM) that is present in all natural waters. In the case of chlorine, it is well known that the total organic halogen (TOX) produced with chlorine is significantly greater than the sum of the halogen mass associated with the well-characterized groups of compounds such as trihalomethanes, haloacetic acids, and haloacetonitriles (Krasner et al., 1989). Moreover, there is a diverse set of nonhalogenated compounds that are formed when water is chlorinated, including formaldehyde, acetaldehyde, and higher molecular weight aldehydes, organic acids, and a host of unidentified peaks on gas chromatographs. However, the halogenated by-products (chlorinated and brominated) that are characteristic of chlorination are the most important to focus on for evaluating similarity, largely because occurrence data are available for some of these DBPs. Other forms of disinfection will give rise to unique by-products, e.g., bromate in the ozonation of water containing bromide ion and

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the formation of chlorite (ClO_2^-) as a by-product of chlorine dioxide (ClO_2). Thus, treatment differences increase the complexity of assessing similarity among different DBP mixtures.

There are substantial gaps in the descriptions of the composition and toxicity of DBP mixtures. A particular problem is the lack of data related to how variations in precursors among water supplies and seasons affect formation of DBP in different chemical classes. This prevents a complete exposition of differences that are likely to occur in water produced in different geographical locations. Serious analytical problems exist for determining whether the data that are available are sufficient to evaluate whether key similarities in DBP mixture composition may be related reliably to the potential for adverse human health effects and in the evaluation of dose response.

This study lays the "groundwork" for evaluating the similarity of mixtures of DBP by (1) describing how variables affect DBP formation, (2) discussing how these factors affect the chemical composition of the mixture, and (3) indicating some of the toxicological implications of differences in composition. Attention is called to those variables that might result in qualitative shifts in the types of DBP that are formed. Such changes represent the most important considerations for assessing the similarity of DBP mixtures. The most critical variables that affect the types and quantities of DBPs that are formed are related to source water qualities, water treatment choices, and distribution system characteristics (Table 1). At low levels of DBPs reported in countries like the United States, changes in conditions including temperature, disinfectant dose, or time that result in small changes in the relative amounts of different DBPs may be of importance for compliance to regulations, but are less likely to reflect substantive differences in the spectrum of health effects observed among DBP mixtures. Other factors, such as bromide levels and types of disinfectants used, are likely to be more influential in altering this spectrum of observed health effects.

The identification of measures that would signal differences in composition is a key step in assessing the similarity of mixtures of DBPs occurring in different water supplies or in the same supply over time. At present, the characterization of the composition of DBP mixtures depends largely upon measures of regulated DBP, which are routinely monitored in the United States. While DBP exposures are associated with a number of effects including reproductive and developmental toxicities, this investigation explores whether there are other measures that might be applied to judge the similarity of DBP mixtures and how these measures might be associated with observed mutagenic or carcinogenic effects.

A subsequent article in this series (Bull et al., 2009a) identifies a number of parameters that may be used to begin the analysis of similarity, focusing largely upon chlorination and chloramination as methods of disinfection. These measures are then evaluated statistically to examine the similarity among a set of mixtures formed by the chlorination of drinking water in different geological and temporal frames (Feder et al., 2009a, 2009b).

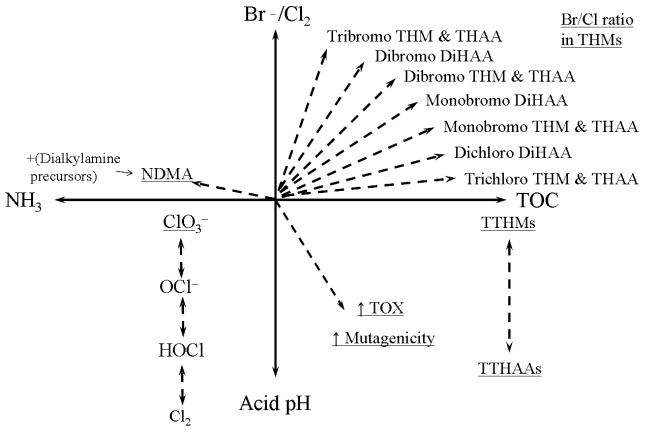
TABLE 1
Factors that Affect the Composition of Disinfectant By-Product Mixtures

Source water characteristics
Total organic carbon (TOC)
Variation in natural organic matter
Season
Temperature
Bromide
Contamination with anthropogenic chemicals
Water treatment and distribution system characteristics
Disinfectant (e.g., chlorine, chloramine, ozone, chlorine dioxide)
Reduction of TOC
Introduction of chemicals that react with disinfectant (e.g., polymers)
Other water treatments (e.g., lime softening)
Time

VARIABLES THAT AFFECT THE NATURE OF DISINFECTION BY-PRODUCTS IN DRINKING WATER

A set of prior reviews (IPCS, 2000; Bull et al., 2001, 2006, 2009a; AWWA, 2008) identified many of the variables that affect DBP formation. In addition, several models were developed to predict formation of the two most studied classes of chlorination by-products, the trihalomethanes (THM) and haloacetic acids (HAA) (Singer, 1994; Amy et al., 1998; Solarik et al., 2000; McClellan et al., 2000). A model was also used to predict the level of mutagenic activity that might be observed in concentrates prepared from chlorinated drinking waters (Koivusalo et al., 1994). Finally, Sadiq and Rodriguez (2004) suggested the use of fuzzy logic for the development of a risk-based indexing system for DBPs. These efforts provide background information and data that are valuable in developing a methodology for evaluating sufficient similarity.

Factors affecting the types and quantities of DBPs formed are presented in Table 1, which identify factors that vary due to differences in source waters and other factors that vary due to differences in water treatment and distribution. Figure 1 places these factors into a conceptual framework that identifies potential differences among mixtures of chlorination by-products occurring in different geographical locations. On each of the axes, one of four factors affecting DBP formation chemistry is displayed as a vector. The vectors illustrate how differences in pH, ammonia, bromide/chlorine ratios, and total organic carbon



NDMA = N-nitroso-N-dimethylamine, TTHM = total trihalomethane, THAA=trihaloacetic acids, THM=trihalomethane, DiHAA=dihaloacetic acid, TOX=total organic halide, TTHAA=total trihaloacetic acids

FIG. 1. Variables that affect formation of chlorination by-products and parameters (underlined) that can assist in determining similarity among mixtures of by-products.

(TOC) affect the composition of DBP mixtures, including the direction of the change in some common measures. For example, as the Br/Cl ratio in the TOX increases, the THM and HAA change from more chlorinated to more brominated species. Another factor is the alkalinity of hypochlorite feed solutions that are frequently used to introduce chlorine. This increased alkalinity may lead to the formation of chlorate (ClO_3^-), which is a subset of the effects of pH; this occurs in the hypochlorite feed solutions rather than in the water being treated. Finally, three measures (i.e., Br/Cl ratio in by-products, TOX, and mutagenicity) are changed by differences in the bulk chemical parameters of bromide/chlorine ratio, pH and total organic carbon (TOC); thus, these bulk parameters also influence the composition of a DBP mixture.

The toxicity of DBP mixtures might vary as changes in these factors alter the types and the quantities of DBP that occur. There are inverse relationships in the concentrations of some DBP classes; in other cases DBP classes appear to co-vary. The formation of certain DBPs is dependent upon very specific precursor inputs to the water treatment plant, and these DBP frequently vary independently from other classes of DBP. To avoid redundancy, the factors that affect the formation of particular DBP are not necessarily discussed in the order shown in Table 1.

Disinfectant Used

The disinfectants used in treating drinking water have a major impact on the nature of the DBPs that are formed. After briefly addressing the complexities of analyzing similarity among DBP mixtures that are formed when combinations of disinfectants are used in the treatment process, this section discusses the differences in the composition of DBP mixtures that are formed when individual disinfectants are used.

As an increasing number of utilities are using combinations of disinfectants, determining the likely composition of DBP mixtures has become more complex. The first disinfectant may react with precursors that give rise to DBP produced by the second. Thus, depletions of this precursor will suppress the concentrations of DBPs associated with the second disinfectant (Gallard & von Gunten, 2002). Conversely, the first disinfectant may create new precursors for the second disinfectant to react with, or, as will be described, the second disinfectant may stabilize intermediate compounds that are produced by the first disinfectant increasing the concentrations of these intermediates relative to more traditional DBPs associated with the first disinfectant. Much of the literature that examines the use of mixed disinfectants has focused on regulated DBPs. The limited mixed disinfectant data are mostly descriptive and not quantitative (Richardson, 1998), and thus not discussed in detail. Studies in actual water treatment plants frequently involve a number of other uncontrolled variables (e.g., differing nature of organic matter in the source water) that make generalization of results imprudent. Therefore, this section confines itself to differences in DBPs that are produced with chlorine, chloramine, ClO_2, and ozone when used independently with model compounds (e.g., fulvic acid) or when in-plant (including pilot plant) comparisons have been made.

Chlorine

Chlorine is added to drinking water as a gas or as a concentrated hypochlorite salt solution. When added to water, chlorine gas reacts with the water forming hypochlorous acid (HOCl) and hydrochloric acid (HCl). As an acid, HOCl dissociates into a hydrogen ion and hypochlorite ion (OCl^-) with a pK_a of 7.5. Because of the differing reactivities of HOCl and OCl^-, by-product formation is affected by pH, as is discussed in a later section.

A major difference in DBP observed with hypochlorite and chlorine gas is that hypochlorite solutions are produced and marketed at an alkaline pH to minimize the volatilization of chlorine gas. At such pHs, OCl^- is converted to ClO_3^- (Bolyard et al., 1993), a reaction that is accelerated by heat. This has two undesirable impacts: It (1) decreases disinfection efficacy, requiring the addition of more hypochlorite solution, and (2) introduces an undesirable by-product to the water, ClO_3^- (NTP, 2005). The resulting ClO_3^- in the treated drinking water was reported as high as 660 μg/L (Bolyard et al., 1993). Gordon et al. (1995) reported median values for ClO_3^- in drinking water disinfected with hypochlorite solutions of 480 μg/L, whereas levels in water treated with chlorine gas were below the limits of detection (10 μg/L) (Bull et al., 2001). To a lesser extent, bromate is formed by the same mechanism when hypochlorite solutions are generated from seawater or sea salt (Weinberg et al., 2003).

The amounts and types of halogenated by-products that are produced by chlorination also depend on its method of addition, source water characteristics, and variables in the distribution

system. The source water characteristics that affect both the quantity and type of by-products formed are NOM (usually measured as TOC), bromide, pH, ammonia, and temperature. The pH and ammonia concentrations in the distribution system also affect the types and quantities of DBPs (Figure 1).

The form of chlorine also interacts with other variables that control formation of particular by-products; other water treatment processes that may be in place frequently modify these variables. For example, since hypochlorite solutions are alkaline and neutralize some of the acidification that occurs with the addition of chlorine gas, these solutions will slightly favor formation of the THM rather than the trihaloacetates or trihaloacetaldehydes as pH varies over between 6 and 8 (Stevens et al., 1989).

Chloramine

Ammonia frequently is added to chlorinated water to reduce the formation of halogenated DBPs. Properly controlling the chlorine to ammonia ratios in the water and maintaining tight control of the pH results in a solution consisting primarily of monochloramine. Monochloramine is less effective as a disinfectant than chlorine, but it is more stable in distribution systems (Kool et al., 1999), and, if properly monitored, may protect against outgrowth of organisms in the distribution system. This fact, coupled with the production of lower THM levels than with chlorine, has increased chloramine's "popularity" in the United States.

Disinfection with monochloramine lowers concentrations of compounds that are trihalosubstituted on a single carbon, such as the THMs, trihaloacetic acids, and trihaloacetaldehydes (Cowman & Singer, 1996). Formation of the dihaloacetic acids generally is not suppressed to the same extent as that of THM and trihaloacetic acids (Hong et al., 2007). This differential effect of chloramine is masked if dihaloacetic and trihaloacetic acids are aggregated into composite measures such as the HAA-5 (monochloro-, monobromo-, dichloro-, dibromo-, and trichloroacetic acid) or HAA-9 (all possible mixed chlorine and bromine HAA). For this reason and because there are distinct differences in their toxicology, it would be helpful from a mixtures research perspective if the individual concentrations of mono-, dihalo-, and trihalo-substituted acetic acid were reported separately. If reporting requirements were to mandate that all haloacetaldehydes are grouped in the same way, it is difficult to characterize risks using this combined measure, because of the great disparity in the mutagenic and carcinogenic properties of mono-, di-, and trichloroacetaldehydes.

Monochloramine also generally reduces the nonpurgeable total organic halogen (NPTOX) (Jensen et al., 1985) relative to that produced by free chlorine. The major difference is that chloramine produces TOX that is more hydrophilic and of a higher molecular weight than formed by chlorine, although this is somewhat dependent on disinfectant dose. However, the chemicals that comprise the TOX depend upon the character of the organic precursors in the treated water. The extent of the difference also differs by the source of the organic carbon that is studied. In a groundwater, the amount of NPTOX was 9% of that produced by chlorine, but ranged from 33 to 49% of that of chlorine when Rhine River humic acids were treated. The TOX produced by reaction of chloramine with fulvic acids from various sources ranged from 12 to 17% relative to reactions with chlorine. On the other hand, the yield of NPTOX from a secondary effluent with chloramine treatment was 37% of that of chlorine. These findings indicate that there may be substantive differences in the yield of halogenated DBP across geographical location due to differences in source water quality.

Recent studies found that *N*-nitrosodimethylamine (NDMA) forms in chlorinated waters when the appropriate precursors are present (CDHS, 1999) (Figure 1). Amounts of this very potent carcinogen found in drinking water are increased in the presence of ammonia (Najm & Trussell, 2000; Choi & Valentine, 2002, 2003; Mitch & Sedlak, 2002a). Formation of NDMA is more substantial in water supplies that have a significant wastewater component, which occurs in many surface water sources. While apparently common in chloraminated supplies at concentrations below 10 ng/L (Fristachi & Rice, 2007), amounts in excess of 1000 ng/L are reported in some wastewaters. Such large differences among water systems result from the presence of dimethylamine or a precursor of dimethylamine in the water that is being treated.

In addition to NDMA, other nitrosamines may form (Mitch & Sedlak, 2002b; Charrois et al., 2004). The major factor in their formation is the availability of an appropriate secondary amine or a precursor to a secondary amine in the source water or from processes used in the treatment plant (i.e., some polymers that are used as coagulants or in anion exchange resins). Therefore, it appears that the presence of dialkylamine precursors is responsible for major variations in dialkylnitrosamine formation between systems, not differences in the nature of NOM. A question that has not been addressed is whether NOM contains alkaloidal secondary amines (e.g., metabolites of tryptophan) that might lead to significant nitrosamine formation. For example, Beiber and Trehy (1983) noted that indoleacetonitrile is a likely product of reactions of phenylalamine with chlorine. Nitrosamines with indoleacetonitrile and several other indole derivatives are also known to form in foods containing nitrite and result in formation of highly mutagenic modifications of DNA bases *in vitro* (Lucas et al., 1999). If any of these precursors are present, the potential for nitrosamine formation is increased with use of chloramine relative to free chlorine for disinfection.

A systematic analysis of probable reaction products of chlorine and chloramine with substructures of NOM found that intermediates in the formation of the THMs and HAAs, specifically halogenated quinones, are likely to be significantly higher if the same water is chloraminated than it would be if it were chlorinated (Bull et al., 2006). Halogenated quinones were shown to be formed with chloramine from phenol and cresol, components of NOM (Heasley et al., 2004). They are generally destroyed by excess free chlorine, in that exhaustive halogenation

destabilizes the quinone ring structure resulting in the formation of HAA and THM. The halogenated quinones are likely to be more stable in chloraminated systems, and this increased stability might be the basis for important toxicological differences among DBP mixtures.

Chlorine Dioxide

The major focus with ClO_2 disinfection has been the potential adverse health effects that might result from exposure to the oxyhalide anions, ClO_2^- and ClO_3^- (AWWA, 2008). ClO_2^- is both a precursor and a by-product of ClO_2. ClO_3^- is generally present at low concentrations in ClO_2 treated water unless hypochlorite solutions are utilized to generate ClO_2 from ClO_2^- (Bolyard et al., 1993).

ClO_2 generally produces less and fewer halogenated by-products than chlorine (Richardson, 1998). In pure ClO_2-treated water THM formation generally is below the limits of detection, although increases in total TOX are observed at about 10% of that observed with chlorine (Chow & Roberts, 1981). However, some halogenated compounds are formed, primarily in the presence of bromide (Zhang et al., 2000). ClO_2 appears capable of oxidizing bromide to hypobromous acid and results in formation of brominated by-products. If ClO_2 is generated with chlorine rather than by acid, some chlorinated by-products could also be formed through reactions with chlorine. In general, the yield of chlorinated by-products when chlorine dioxide is generated by activating chlorite with chlorine is lower than would be encountered with chlorine alone (Bull et al., 2009b).

Ozone

Ozone (O_3) acts primarily as an oxidant. Consequently, it produces relatively high concentrations of nonhalogenated organic aldehydes, ketones, and acids (Koga et al., 1991; Krasner et al., 1993, 2006; Richardson et al., 1999). The compounds most frequently measured are formaldehyde and acetaldehyde, but higher molecular weight aldehydes also are formed (Glaze et al., 1989). Generally, the concentration of these aldehydes is higher in ozonated water than in chlorinated waters.

Ozone indirectly halogenates organic precursors by oxidizing bromide, which occurs naturally in source waters, to hypobromous acid, which then reacts to form brominated DBP (Najm & Krasner, 1995). Consequently, brominated DBP are frequently observed in ozonated drinking water, although generally at concentrations that are below those observed with chlorine in the same water (Amy et al., 1998).

The major concern identified with ozonated water is bromate (Najm & Krasner, 1995). The reactions by which ozone produces bromate from bromide are complex. Simply, they may be viewed as oxidation of bromide to hypobromous acid, a dissociation of hypobromite ion, and further oxidation to bromate. The latter reaction is favored at alkaline pH (von Gunten, 2003).

Ozone is invariably used with a secondary disinfectant in the United States, most commonly chlorine or chloramine. Unfortunately, systematic studies of the relative yields of DBP of real interest are rare. Miltner et al. (1992) found that total THM concentrations were reduced when ozonation preceded chlorination. However, the concentrations of dibromochloromethane and bromoform increased while the total trihalomethane (TTHM) concentrations declined because chloroform and bromodichloromethane (BDCM) concentrations declined. This dynamic was attributed to an O_3-induced decrease in TTHM precursors in the low bromide system they studied. On the other hand, preozonation increased chloropicrin formation more than fourfold. This was attributed to an increase in the precursors of this DBP (and presumably for its brominated analogs) by preozonation. In a more recent study (Richardson et al., 2008), concentrations of some halogenated DBP, specifically dichloroacetaldehyde, 1,1-dichloropropanone, 2-chloro-2-methyl propanol, and 2-bromo-2-methylpropanol, were substantially higher in water that was preozonated relative to water treated with chlorine or chloramine alone. These findings are entirely consistent with the predictions of Reckhow and Singer (1985) that preozonation would be expected to decrease TOX, increase the formation of halopropanones, and shift formation of THM and trihaloacetic to dihaloacetic acids. Obviously, the patterns of these less abundant by-products differ from those of the THM, and there are important issues of changing composition among the HAA. Based on these data, regulated DBPs appear to be poor surrogates for the formation of these less frequently measured halogenated aldehydes, ketones, and propanols when O_3 is used in combination with chlorine or chloramine.

Natural Organic Matter

TOC concentration is one of the main determinants of the amount of organic by-products formed through chlorination. In general, source water TOC levels are positively correlated with the amounts of recognized by-products such as the THMs, HAAs, chloral hydrate, and TOX (Singer, 1994; Singer et al., 1995; Amy et al., 1998).

On the other hand, it is important to recognize that the composition of the NOM varies significantly among water sources and temporally within the same source (Krasner et al., 1994, 1996, 2006). Leenheer et al. (2000, 2007) showed changes in the distribution of NOM fractions in natural waters, in waters impacted by advanced wastewater treatment plants, and in wetlands. Rosario-Ortiz et al. (2007) evaluated the characteristics of four separate tributaries to Lake Meade finding substantial differences in the chemical nature of NOM. They found that the variation among these waters depends largely upon the extent to which the water was influenced by microbial activity. Rodriguez et al. (2004) demonstrated fivefold changes in TTHM concentrations and fourfold changes in HAA concentrations in the same water supply over seasons. While the changes reported by Rodriguez and collaborators (2004) are

unusually large, they underscore the influence of differences in the NOM. Most importantly, Nikolaou et al. (2004) found that the seasonal variations in the concentrations of minor by-product classes (e.g., halopropanones, chloral hydrate) differed from those of the TTHM and HAA.

The nature of halogenated by-products displays a dependence upon whether the water is drawn from a flowing stream versus a lake or reservoir. In the 35-city study (U.S. EPA, 1989), this led to a complete decoupling of the relative yields of THMs and some of the minor by-product classes (Bull et al., 2009a).

The organic matter from different sources varies in the amount of nitrogen it contains. Therefore, some waters yield significant quantities of nitrogenous DBPs. The occurrence of haloacetonitriles (HAN) was studied more extensively than other nitrogenous DBPs. Their yields differed substantially among 35 water utilities, with mean annual averages from 0.3 to approximately 15 μg/L (U.S. EPA, 1989). Changes in other nitrogenous DBPs are likely to display similar differences among different water sources.

Some nitrogenous compounds enter drinking-water sources in treated waste waters. The formation of nitrosamines is a case in point. Chloramine may react with nitrogenous compounds reported in wastewaters such as dimethylamine, other dialkylamines, pyrrole, pyrrolidine, morpholine, or their precursors, forming substantive amounts of the corresponding nitrosamines (Mitch & Sedlak, 2002a, 2002b; Charrois et al., 2004).

When water containing high amounts of municipal wastewater is used as a drinking-water source, the concentrations of HAA may exceed the TTHM concentrations by a factor of 3. In contrast, source waters containing lower levels of municipal wastewaters usually yield total haloacetic acids (THAA) at or below the TTHM concentration (NRC, 1998).

Analyses of some available survey data suggest that qualitative differences in the nature of the organic carbon present influences the makeup of the mixture of halogenated by-products in substantive ways (Bull et al., 2008a). However, variations in the relative yields of different classes of halogenated by-products relative to differences in the nature of the organic carbon have not been studied systematically.

Bromide and Iodide

Bromide ion in water as it is chlorinated results in the formation of brominated by-products. As these concentrations increase, brominated compounds can eventually dominate the by-products that are formed (Krasner et al., 1989). Brominated by-products are formed before chlorinated ones because chlorine reacts preferentially with bromide to form hypobromous acid (Amy et al., 1985). Hypobromous acid more efficiently halogenates compounds than HOCl. The formation chemistry of iodinated compounds is similar if the treated water contains iodide (von Gunten, 2003). However, iodinated by-products occur infrequently and are generally present at much lower concentrations than brominated by-products.

The 35-utility survey demonstrated the marked effects that high bromide concentrations exert on the mixture of DBP (Krasner et al., 1989). The distribution of chloro, bromochloro, and brominated THM is provided in Table 2. While TTHM concentrations in the waters from the treatment plant that had the highest bromide concentration were higher (≥40 μg/L) than those in waters from the other plants (≤30 μg/L), the chloroform concentrations were consistently less than 1 μg/L. In contrast, the chloroform concentrations in the systems with lower bromide were ≥10 μg/L.

All classes of halogenated organic DBP increase their bromine relative to chlorine content as the inorganic bromide concentration rises in the treated water. This was demonstrated with the formation of the THM, HAA, HAN, haloaldehyde, haloketone, and halofuranone groups of disinfection by-products (Krasner et al., 1989, 2006; Amy et al., 1994). The degrees of bromination of the potent mutagenic analogs of 3-chloro-4-(dichloromethyl)5-hydroxy-2-(5*H*)-furanone (MX) were also shown to shift systematically with the THMs as bromide concentrations increase (Suzuki & Nakanishi, 1995). Because bromination is a faster reaction than chlorination, the TOC concentration also influences the relative amounts of brominated versus chlorinated by-products (Shukairy et al., 1995). This can be viewed most simply as a competitive saturation of halogenation sites on a precursor molecule by bromine that limits chlorination. Thus, a bromide to TOC ratio that is large will lead to a greater proportion of brominated by-products in chlorinated drinking water.

In summary, the presence of bromide in water treated with chlorine produces increasing amounts of brominated organic by-products. This shift in chemistry is observed in all classes of halogenated organic by-products. The relative yield of brominated species also depends upon the bromide to carbon ratio and the bromide to chlorine ratio. Increases in that ratio, either

TABLE 2
Influence of Bromide Concentration on the Distribution of Trihalomethanes (THMs)

THMs	Summer	Fall	Winter
All systems			
Chloroform	15	13	9.6
Bromodichloromethane	10	5.5	4.1
Dibromochloromethane	4.5	3.8	2.7
Bromoform	0.57	0.88	0.51
High bromide system			
Chloroform	0.95	0.59	0.72
Bromodichloromethane	3.8	2.9	4.1
Dibromochloromethane	8.6	9.2	11
Bromoform	30	40	31

Note. From Krasner et al. (1989).

by rising amounts of bromide in the presence of the same TOC concentrations or as a decrease in TOC as a result of precursor removal with a constant amount of bromide, result in substantial shifts in the relative amount of brominated by-products that result from chlorination. The presence of bromide in the water being treated also is responsible for the formation of brominated organics DBP with O_3 and ClO_2. In the case of O_3, bromate is also produced by oxidation of bromide in the treated water.

pH

pH modifies chlorination by-product character in complex ways. Figure 2 illustrates the magnitude and character of changes observed in the formation of TOX, chloroform, and trichloroacetic acid (TCAA) when chlorine is reacted with fulvic acids at varying pH (Reckhow & Singer, 1985). The organic carbon is most efficiently halogenated at pH below the pK_a of HOCl. In addition, some halogenated by-products are labile at alkaline pH. Therefore, net formation of TOX progressively decreases as pH increases. TCA formation is maximized at pH 5, and it progressively decreases with increasing pH. In contrast, chloroform formation is enhanced at high pH. The production of the other THM parallels the chloroform data in actual drinking water (Summers et al., 1996). Like TCA, trihaloacetaldehydes with substitutions on a single carbon are labile under acid conditions (Stevens et al., 1989). The trihaloacetic acids and aldehydes are decarboxylated to form THM. In contrast, dihalogenated acetic acids are quite stable to variations in pH (Stevens et al., 1989; Summers et al., 1996). Most important is that the TOX exceeds the total measured DBP concentrations, highlighting that much of the TOX has not been accounted for by known DBP, particularly at acid pH.

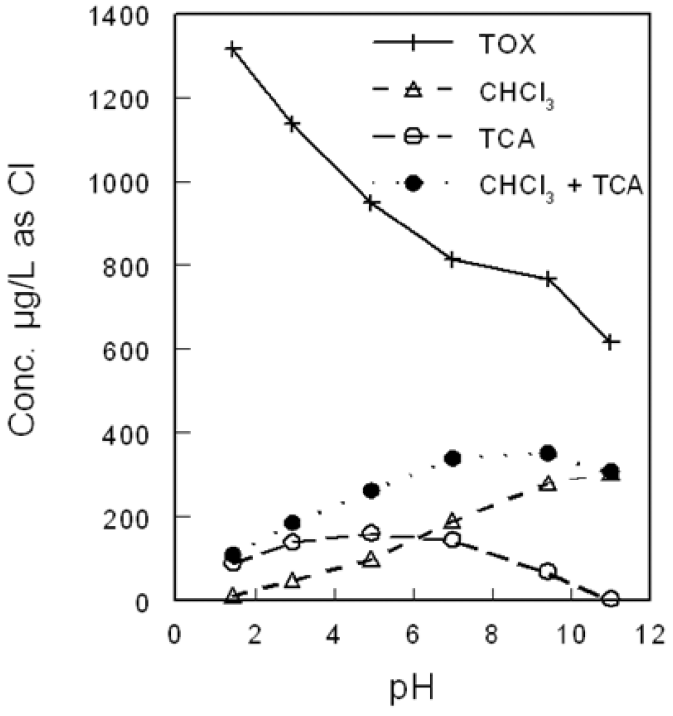


FIG. 2. The effect of pH on formation of chlorination by-products from fulvic acid. Conditions involved addition of 20 mg/L HOCl to 4.1 mg TOC/L. Reaction time was 72 h. Adapted from Reckhow and Singer (1985).

In addition to these examples, other by-products are labile under alkaline conditions. MX, in particular, is much more efficiently formed at acid pH and virtually none can be detected if the chlorination is conducted at pH 9 (Backlund et al., 1989). Part of this difference is due to the isomerization of MX to an open-ring form (*Z*-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid, commonly referred to as E-MX) as pH increases. The pK_a at which this occurs is 5.25, where both forms are present at equal concentrations. Above this pH, E-MX predominates. Other haloaldehydes, haloketones, and halonitriles are also labile at alkaline pH (Bieber & Trehy, 1983; Croué & Reckhow, 1989). Therefore, differences in pH within the range commonly found in drinking-water systems around the country (usually considered to be between 6 and 9 although some more extreme pH can be encountered) may exert substantial effects on the mixture of DBP that are found in drinking water.

The speciation of chloramines and bromamines are influenced by pH. The addition of ammonia to chlorine results in the formation of a mixture monochloramine, dichloramine, and trichloramine. The relative concentrations of these forms depend upon the ratio of chlorine to ammonia and pH. Trichloramine is almost 1000-fold more volatile than monochloramine and a strong respiratory irritant. As a result if the pH of the water falls below 8 the portion of the chloramines that exists as trichloramine progressively increases and quickly volatilizes. It is odoriferous, so its formation is avoided by keeping the pH at approximately 8.3. A chronic respiratory irritation has been associated with the use of chloramine in food processing and indoor swimming pools (Hery et al., 1995). The same general rule applies to formation of the bromamines. To maintain monochloramine as the predominant form, the pH needs to be slightly alkaline (maximum formation occurs at pH 8.3) and more even more alkaline (pH 9) to minimize the concentrations of tribromamine (Issac et al., 1985).

As indicated earlier, pH also influences the relative amount of brominated DBP formed with O_3. In this case, the formation of bromate is favored at alkaline pH, whereas brominated organics are produced at neutral to acid pH (von Gunten, 2003).

Temperature

The effect of temperature on DBP formation has been viewed as simply related to higher reaction rates. There has been little investigation of by-product classes other than the THM in this regard. In general, THM formation increases with temperature (Oliver, 1980). Interestingly, the proportion of the

THM that are brominated increases with decreasing temperature. It is also important to indicate that those DBP that degrade in the distribution system also are likely to degrade at a more rapid rate with higher temperatures, although no studies were identified that establishes the temperature dependence of their decay.

The effect of temperature on by-product formation is obviously confounded by seasonal variations in the nature of NOM (Leenheer et al., 2007). This is a potentially important distinction because the seasonal variation in NOM found in surface water sources also may contribute to differences in DBP yields (Bull et al., 2009a). Clearly, the processes producing NOM (e.g., reactions that occur in decaying vegetation) on a particular watershed at different times of the year are variable in part due to differences in temperature as well as differences in the amount of fresh vs. older foliage and microbial activity at different times of the year (Leenheer et al., 2007). In the case of lakes and reservoirs, the quality of the source water often changes from winter to summer by upset of thermoclines that turn over lower layers that may contain NOM of differing ages.

Water Treatment and Distribution

Processes involved in water treatment influence the amount and nature of DBP observed at the tap. For the most part, conventional treatment processes remove precursors of DBP rather than the DBP themselves. Sohn et al. (2007) examined the nature of organic carbon at different points in a drinking-water treatment plant. Coagulation, ozonation, and biologically activated carbon (BAC) incrementally decreased the formation of the THMs and HAAs. Similarly, granular activated carbon and reverse osmosis treatment effectively remove precursors of the THM and HAA (U.S. EPA, 2001a). The effects of such treatments on removing precursors of other DBPs have not been evaluated directly.

The presence or absence of a residual disinfectant in the distribution system may alter the DBP mixture. For example, the dihaloacetic acids and dihaloaldehydes are susceptible to microbial degradation (Baribeau et al., 2000). Therefore, their concentrations may be decreased by microbes resident in the distribution system (Schenk et al, 2008), while other DBP, such as THM, are observed to increase due to prolonged reaction time (Rodriguez et al., 2007).

Time

Drinking waters in contact with residual disinfectants for long periods of time continue to form DBP. Many utilities predisinfect water as it enters the treatment plant. Disinfectant in excess of demand (chlorine or chloramine) is usually added as the treated water leaves the plant (in the United States), producing a residual to protect against microbial contamination and re-growth in the distribution system. The residence times to various locations in the distribution system ranges from hr to days (U.S. EPA, 2001a). The presence or absence of residual disinfectant results in changes in the concentrations of DBPs as the water is being distributed. There are various reasons for such changes, ranging from the instability of some by-products, to degradation of others by microbes that colonize distribution systems, or continued reaction with the more slowly reacting precursors that remain in the water (Reckhow & Singer, 1985).

Figure 3 illustrates some of the changes in composition that may occur in DBP mixtures over time. These data were collected following addition of excess HOCl to a solution of fulvic acids at pH 7 and following the concentrations of several by-products for 320 h (Reckhow & Singer, 1985). While TOX, THM, and HAA tend to increase with time, minor by-products, represented by 1,1,1-trichloropropanone (1,1,1-TCP) and dichloroacetonitrile (DCAN), decreased with time and virtually disappeared over the course of the experiment (Reckhow & Singer, 1985).

1,1,1-TCP is one of several precursors of chloroform. This pathway, a minor source of chloroform, results from the relatively slow conversion of 1,1,1-TCP to chloroform that occurs in the presence of excess chlorine over time (Reckhow & Singer, 1985). Similarly, DCAN slowly decomposes to dichloroacetamide and then to dichloroacetic acid. The kinetics of the reactions of formation and decomposition of DBPs suggest that the exposure of individuals to these less abundant by-products are likely to differ significantly within the same water system. It is important to consider the possibility that if the toxicological potencies of some minor by-products are greater than those of the major by-products, then these minor by-products will contribute significantly to the differences in toxicity among DBP mixtures in proportion to their potency (Bull et al., 2006).

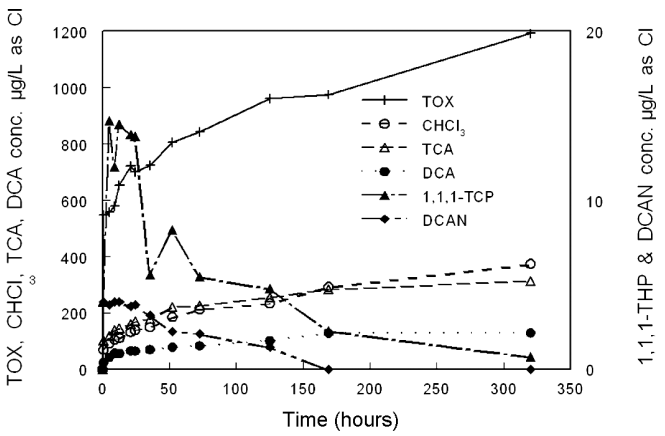


FIG. 3. Formation of chlorination by-products from fulvic acid with time. The experiment involved treatment of fulvic acid at a concentration of 4.1 mg TOC/L that was treated with 20 mg/L of applied HOCl at zero time. Adapted from Reckhow and Singer (1985).

HEALTH EFFECTS DATA THAT BEAR ON THE SIMILARITY AMONG DBP MIXTURES

Existing toxicological and epidemiological data on DBP mixtures and individual DBP may contribute to an evaluation of the similarity of DBP mixtures. In chemical mixtures risk assessment, there are two general approaches commonly used to estimate the body's response to a multiple chemical exposure: component-based approaches and whole-mixture approaches (U.S. EPA, 2000). Component-based approaches rely upon individual chemical toxicity data and an understanding of how the chemicals harm human health collectively, whereas whole-mixture approaches rely on toxicity information about a mixture that was evaluated and how similar the encountered mixture is to the tested mixture (U.S. EPA, 2000). The toxicological similarity of a group of mixtures may be evaluated by appropriate testing of a whole mixture and also through an evaluation of the toxic properties of its components (Rice et al., 2009).

Ideally a comprehensive health effects database for individual DBPs and whole mixtures would serve as the basis for making confident judgments regarding the similarity of mixtures. In the ideal database, the toxicology of all of the DBPs would be well understood and the toxicological and epidemiological results would be supportive and mutually consistent. Such a database would greatly simplify the evaluation of similarity among DBP mixtures that were well characterized chemically.

In reality, significant data gaps exist between the ideal and the current state of knowledge. Epidemiological and toxicological data on whole DBP mixtures is limited. There also are inconsistencies between the epidemiological and toxicological literature. Some epidemiological studies purported to show associations between exposures to DBP mixtures, classes of DBPs, or specific DBPs and increased risks of some cancers. While consistent epidemiologic associations with entire mixtures may be difficult to dismiss, associations with individual components of the mixture are more tenuous because so much of the mixture remains chemically and toxicologically uncharacterized. On the other hand, toxicological tests on individual DBPs and subset mixtures have been conducted generally at levels significantly higher than the current low levels found in U.S. drinking waters. The high-dose test results for this small number of individual DBPs (relative to the hundreds of identified DBPs) suggest these might only partially account for the risks reported in positive epidemiological studies (Bull et al., 2006) (i.e., the toxicological tests conducted to date on the DBP regulated in the United States suggest that their toxicological potency at environmental exposure levels is modest). This suggests that if the positive epidemiologic studies accurately assess the magnitude of the effects, then the remaining DBPs that have not been characterized chemically or characterized toxicologically must account for much of the mixtures toxicity.

Figure 4 places the changes in chemistry into the framework of what is known about the toxicology and epidemiology of DBP mixtures. This combination of information is far from exhaustive, but it shows how such information can be drawn on to begin to evaluate similarity among DBP mixtures. In broad terms, toxicological methods can be applied in the form of bioassays of representative samples of the whole mixture or they can be utilized to provide data for evaluating the contribution of individual components or component classes that might make the mixture unique. Epidemiological studies have generally

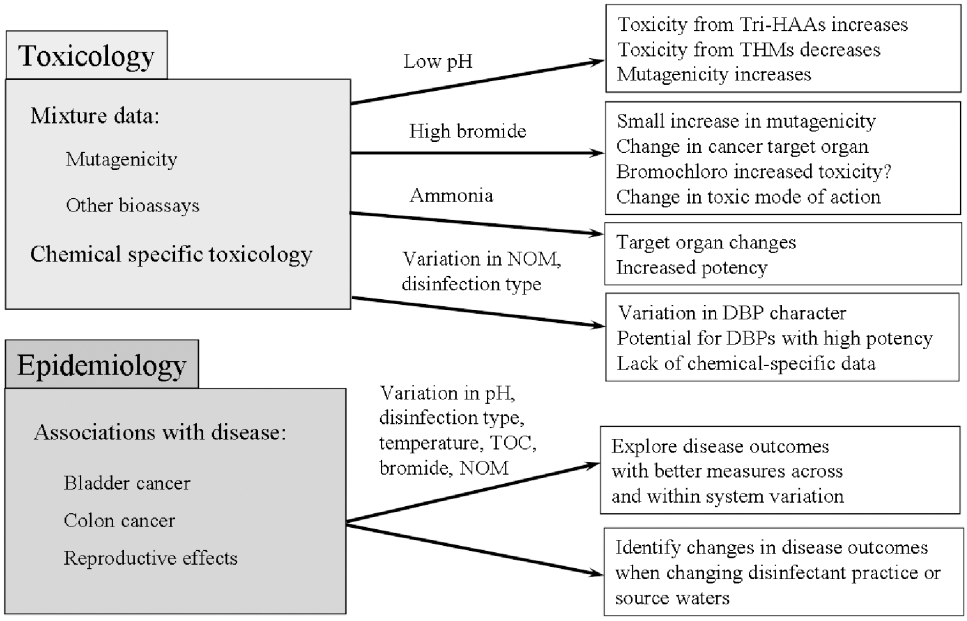


FIG. 4. The role of health effects data in identifying source water and treatment variables that differentiate DBP mixtures.

evaluated effects that may be associated with the whole mixture, but also can be utilized to determine if differences in disinfection practice or water sources significantly alters the associations observed with drinking waters. The contributions these studies can make in assessing the similarities among mixtures are discussed in turn in the following sections. The remaining sections of this article focus on the potential use of studies describing the mutagenic activity of DBP mixtures and DBP cancer epidemiology and toxicology studies to evaluate similarity among DBP mixtures.

Mutagenic Activity of Mixtures of DBP

Bioassays of organic fractions isolated from drinking water have been conducted for more than 30 years (Loper et al., 1978; Bull et al., 1982; Cognet et al., 1987). The biological systems that have been applied vary widely in sensitivity and specificity. Some of these toxicological test systems provide both qualitative and quantitative information that might be useful for assessing differences among DBP mixtures. *In vitro* assays are useful if they address very specific biological effects (e.g., mutagenicity). However, *in vitro* tests lack important determinants of toxicological potency (e.g., factors controlling absorption, distribution, metabolism, excretion). They also provide no information on other endpoints that may result in adverse health effects. Despite these limitations, this investigation focuses on the use of *in vitro* bioassays as another measure that might be applied to the evaluation of the similarity of different DBP mixtures. It is beyond the scope of this study to evaluate the use of all of the different test systems that have or could be applied. Therefore, this investigation focuses on the consideration of how mutagenicity testing using *Salmonella* tester strains, which has the capability to identify a specific biological effect that results in adverse health effects, might be applied to judgments regarding the similarity of DBP mixtures. This assay is well established and has a number of variants that provide diagnostic information. Mutagenicity testing of DBP mixtures using *Salmonella* tester strains was studied consistently over the last 30 years (Meier, 1998; Richardson et al., 2007; Claxton et al., 2008).

Mutagenic Activity With Use of Alternative Disinfectants

Chlorine

Increased mutagenic activity is invariably associated with chlorination (Meier, 1990). Much of this activity may be attributed to the formation of MX and related compounds (Meier et al., 1987). Levels of mutagenic activity produced by chlorine are strongly dependent upon pH (Figure 1). This is particularly true of MX (Zhuo et al., 2001). MX formation kinetics differ from those of more commonly measured chlorination by-products (e.g., DCA, TCA, chloroform). While there are correlations that are observed, the coefficients are low (e.g., 0.633 [Vartiainen et al., 1988] and 0.67 [Schenck et al., 2008] with chloroform). Therefore, mutagenic activity may play a role as a substitute measure for MX and related compounds that are challenging to measure at concentrations commonly found. The study of Wright et al. (2002) appears to support this idea as seasonal patterns of mutagenicity and MX concentrations were positively correlated, while the seasonal pattern of THM formation was quite different.

The reasons for the focus on strains TA98 and TA100 are illustrated by the systematic study of mutagenic activity that is generated by reaction of chlorine with humic acid (Meier et al., 1983). Chlorination induced concentration-related increases in mutagenic activity in the absence of metabolic activation in strains TA1535, TA1537, TA1538, TA98, and TA100. In general this activity was decreased by the addition of the supernatant from an S9 fraction of a liver homogenate. The maximum response observed with TA100 was an increase of approximately 800 revertants/plate at the highest concentration of humic acid, 110 revertants/plate for TA98, 45 revertants/plate with TA1537, 30 revertants/plate with 1535, and 5 revertants/plate with TA1538. Only because of its larger net response has TA100 become the "standard" tester strain applied to drinking-water samples. However, these strains are designed to detect different types of mutagenic events; other strains were subsequently developed to detect mutagens and differentiate among the mechanisms by which mutations are produced. The use of simple mutagenicity assays to detect more subtle differences in DBP composition requires modification of the approach of using one or two *Salmonella* tester strains as the main method of evaluation.

The mutagenic activity measured in chlorinated drinking water with *Salmonella* TA98 or TA100 tester strains includes a large contribution from halogenated furanone derivatives (Meier, 1988) and minor contributions from other compounds, including haloaldehydes, halonitriles, and haloketones, formed at higher concentrations (U.S. EPA, 2002). The activity in chlorinated water is generally observed in the absence of exogenous metabolic activation. While weak mutagenic activity is found among members of more abundant classes of chlorination by-products such as the THMs, HAAs, and HANs, their activity appears to contribute minimally to the total mutagenic activity of chlorinated drinking water as measured in *Salmonella* TA100 (Meier et al., 1985).

Organic *N*-chloramines represent a neglected group of mutagenic chemicals that may be present at relatively high concentrations (i.e., >100 μg/L) based on an estimated median concentration of dissolved organic nitrogen in surface waters of 350 μg/L (Bull et al., 2008a). *N*-Bromamines also can be formed, but are considerably less stable than organic *N*-chloramines at pH typical of drinking water (Issac et al., 1985). Although mutagenic (Thomas et al., 1987), these chemicals have received little further toxicological characterization. This is largely because the specific nature of the *N*-chloramines that occur in drinking water has not been established.

The precursors for the *N*-haloamines in natural waters are diverse. Free amino acids are estimated to comprise about 6% and combined amino acids (i.e., polypeptides and proteins) about 12% of the total dissolved nitrogen and received most attention (Bull et al., 2008a). *N*-Chloramines also are formed with the bases of nucleic acids and amino groups within humic acids. However, these recognized biochemicals represent only about 41% of the total dissolved nitrogen. The *N*-haloamines of many amino acids are oxidized by free chlorine to form the corresponding organic acid (e.g., alanine → *N*-chloroalanine → pyruvic acid) (Jensen et al., 1985). The initial *N*-chloramines are more stable when chloramine is used as the disinfectant. This differential in occurrence of organic *N*-chloramines might be expected of certain non-amino acid precursors of organic *N*-chloramines as well.

Recently, high levels of mutagenic activity were associated with halogenated nitromethanes, especially the mono- and dibromonitromethanes (Plewa et al., 2004), but these chemicals are not consistently observed even in the same water (Krasner et al., 2006) and, when detected, are generally found at concentrations below 1 μg/L. The only nitrohalomethane tested as a carcinogen is chloropicrin and there was no evidence of carcinogenicity (NCI, 1978).

The overwhelming mutagenic activity of MX in the strains usually used (TA100 and TA98) masks substantial changes in the concentrations of weaker mutagens. This is illustrated by the rather small variations in mutagenic activity (less than a factor of 2 difference) despite rather wide variations in chemical composition within humic and fulvic acid fractions obtained from diverse sources (Watt et al., 1996). Perhaps further studies that associate specific mutagenic events with mutagens other than MX that also are produced by chlorination may make these tests more diagnostic of similarity among mixtures (DeMarini et al., 1995; Hyttinen et al., 1995; Knasmüller et al., 1996; Richardson et al., 2007).

Tennant et al. (1987) noted that the mutagenicity of chemicals did not correlate well with cancer potency estimates. The cancer slope factor estimates associated with weak mutagens may be similar to those of potent mutagens. For example, MX is potently mutagenic, but only moderately carcinogenic.

Chloramine

The use of chloramine in disinfection of water results in increases in mutagenic activity, but to a lesser extent than chlorine (DeMarini et al., 1995). Largely, this reflects a reduction of MX concentrations (Wright et al., 2002). This conclusion is supported by the similarity of mutation spectra obtained from *Salmonella* TA100 in extracts of water treated with chloramine versus chlorine (DeMarini et al., 1995).

While use of chloramine generally reduces the formation of total halogenated chemicals (Vartiainen et al., 1988), not all by-product classes are affected the same way. As indicated earlier, organic *N*-chloramines are produced with both chlorine and chloramine. However, free chlorine tends to degrade many of the organic *N*-chloramines, whereas chloramine residuals tend to stabilize these chemicals (Jensen et al., 1985). Monochloramines form rapidly with alpha-amino acids, but dichloroamines are formed preferentially with amines that are not in an alpha position with a carbonyl (e.g., ethanolamine or the epsilon nitrogen of lysine). Dichloramines convert more readily to free radicals that damage macromolecules (Nightingale et al., 2000) and generally possess higher mutagenic activities than monochloramines. As a consequence, they are more likely to be of health concern. Although these compounds could be present at much higher concentrations, their mutagenic activity is low compared to MX (Thomas et al., 1987).

One chemical that is known to increase in chloraminated (chlorine + ammonia) water relative to simple chlorination is NDMA. While NDMA was shown to be mutagenic under special experimental circumstances (suspension assay) or in specifically engineered *Salmonella* strains such as the acetyltransferase-overexpressing *Salmonella* strain NM2009 (Yamazaki et al., 1992), it is not active in the plate assays that were applied to surveys of drinking-water treatment (Bartsch et al., 1976).

Therefore, it is probable that the lower mutagenic activity associated with chloramine disinfection when compared to chlorine disinfection largely reflects a reduction of the MX concentration. However, chloramine disinfection produces increased concentrations of other genotoxic compounds (e.g., nitrosamines) that were not detected in the standardized plate assay.

Chlorine Dioxide

Mutagenic activity was associated with ClO_2-treated water. However, it is difficult to compare these results with chlorination because these studies were conducted using different methods of sample preparation and different measures of genotoxic activity. Mutagenic activity produced by ClO_2 is generally much lower than observed with chlorine (Kool & Hrubec, 1986). When chlorine is used to activate chlorite to produce chlorine dioxide, the mutagenic activity also is lower than that observed by the use of chlorine alone, but it appears to be largely attributed to MX (Kronberg & Christman, 1989). This suggests that the mutagenic activity produced with the use of ClO_2 is largely attributable to free chlorine.

Ozone

Ozone produces mutagenic by-products with short contact times, but these seem to be destroyed with contact times that are typically utilized in drinking-water treatment (Kool & Hrubec, 1986). However, when O_3 is used as the primary disinfectant, it is usually followed with chlorine or chloramine as secondary disinfectants. This results in substantive increases in the mutagenic activity that is measured with strains TA98 or TA100 compared to O_3 alone. In these cases, the mutagenic activity observed is less than with chlorine or chloramine alone (approximately 40 and 85% respectively)

(DeMarini et al., 1995). However, the mutation spectra that are observed is again consistent with a predominate influence of MX or related mutagenic DBPs.

There appears to be a much greater likelihood that O_3 produces mutagenic by-products if the treated water is impacted by domestic wastewater. In the case of water that was variably impacted by wastewater effluents because of seasonal changes in flow, quite variable contributions of ozonation to mutagenic activity were observed (Dolara et al., 1981). This system utilized prechlorination and a terminal treatment with O_3. At certain times of the year, this apparently produced high levels of mutagenic activity that was dependent upon metabolic activation, a substantive departure from what is observed with chlorine. An earlier study of the mutagenicity of ozonated, recycled water (Gruener, 1978) also found that the bulk of the mutagenic activity was observed only after the introduction of liver microsomes from animals pretreated with inducers of xenobiotic metabolism. These studies suggest that a broader analytical approach to mutagenesis testing might provide useful information about differences among DBP mixtures.

Altered Mutagenic Activity With Bromide

The bacterial mutagenic activity present in drinking water might be expected to vary depending upon bromide concentrations. Mutagenic activity in the absence of metabolic activation was significantly increased when bromide was present when chlorine was added to humic acid (Meier et al., 1985). The rise was modest, approximately doubling the response. Moreover, the mutagenic activity peaked at different bromine to chlorine ratios depending upon whether *Salmonella* tester strain TA100 (0.05:1) or TA98 (0.1:1) was used. This overall increase in mutagenic activity is consistent with the substantially higher mutagenic activity of brominated aldehydes versus chlorinated aldehydes (Rosen et al., 1980; Eder et al., 1982; Segall et al., 1985) and ketones (Hussain & Osterman-Golkar, 1984) that are similar to those produced in the chlorination of drinking water. In these cases the mutagenic potency increases as much as 10-fold with bromine substitution. Brominated analogs of MX, however, are not consistently more potent than the chlorinated forms (Suzuki & Nakanishi, 1995; LaLonde et al., 1997). In part, this could be due to complex interactions with a neighboring hydroxyl group by multiple halogens as this hydroxyl group is a major determinant of mutagenic activity of MX (LaLonde et al., 1991a, 1991b; LaLonde & Leo, 1994).

A range of 15–60% of the mutagenic activity produced by chlorination was attributed to MX (Meier et al., 1987). This estimate did not correct for recovery. Recent analytical studies indicate that MX is present at substantially higher concentrations than was measured in earlier studies (Krasner et al., 2006). Moreover, significant additional contributions also may be expected from the monobromo analogs, which are present even at low bromide concentrations (Suzuki & Nakanishi, 1995). Despite the overwhelming importance of MX and its analogs in determining specific mutagenic activity, relatively small changes in mutagenic activity are expected as a result of a shift to its brominated analogs. Therefore, relatively large changes in the concentrations of brominated analogs of the haloaldehyde and haloketone classes might result in only small changes in mutagenic activity relative to the high activity of MX (Meier et al., 1987, vs. Meier et al., 1985). As a result, mutagenic activity may not be a sensitive measure of the similarity (or more properly dissimilarity) primarily when precursors of DBPs shift away from naturally occurring organic matter to synthetic precursors that appear in domestic wastewaters.

Variation of Mutagenic Activity With pH

Since a predominant bacterial mutagen in chlorinated drinking water is MX, it is not surprising that the mutagenic activity of chlorinated drinking water varies significantly with pH. It is also important that mutagenic activity be measured in samples that have been collected and preserved at an acid pH, rather than at the existing pH of the finished drinking water, to obtain accurate measurements of the amount of MX that is present. While there are other mutagens present (Meier, 1990), their relative potency is low and it is difficult to assess the meaning of variations in mutagenic activity without more specific information on the chemical nature of the mixture.

UTILIZING EPIDEMIOGICAL DATA TO INFORM THE ASSESSMENT OF SIMILAIRITY AMONG DBP MIXTURES

Carcinogenicity associated with mixtures of chlorination DBP is of particular interest because of small, but consistent associations of cancer with chlorinated water in epidemiological studies, in particular cancer of the bladder (U.S. EPA, 2003). Cancers in other sites such as colon, rectum, or brain also have been associated with exposures to chlorination DBPs, but much less consistently. It is not clear whether the variations among these other cancer sites are spurious or actually reflect differences in DBP mixtures across geographical locations and differences in source water quality.

The available epidemiological data on DBP mixtures have little ability to distinguish the relative importance that individual DBPs might have in the development of adverse health effects. Typically, only a small subset of the DBPs is measured in these studies and the exposure gradients reported can be quite narrow. In addition, determining independent effects of highly correlated variables is difficult due to co-linearity in statistical models. Since epidemiological studies cannot easily provide discriminators among chlorinated water supplies, it is necessary to utilize the limited toxicological data that are available to determine whether some measures might be effective discriminators.

As noted previously, there are marked differences between the magnitude of effect estimates reported in some epidemiological studies and the dose-response slopes estimated from rodent bioassays of the common DBPs. For example, epidemiological

associations of the THMs with cancer suggest risks significantly greater than can be derived when the dose-response estimates that are based on the available toxicological on these compounds is combined with the DBP doses that occur in chlorinated drinking water In other words, the carcinogenic potency of THMs in toxicological studies might account for a small fraction of the cancer risk estimated from the epidemiological data (U.S. EPA, 2003). Moreover, BDCM, when administered in drinking water, was recently shown to be negative for carcinogenic activity in an NTP (2006b) bioassay. Finally, none of the THM were found to target the urinary bladder in controlled animal studies, nor do their structures resemble those of compounds that are known bladder carcinogens (IARC, 1999). Therefore, great uncertainty exists in the use of the THMs as surrogates for carcinogenic risk when assessing similarity among DBP mixtures.

Despite its limited ability to discriminate among potential causal agents in a complex mixture, epidemiology has helped and can help elucidate cancer risk relative to various water treatments. For example, case control studies found that chlorinated water was associated with an increased risk of bladder cancer (IARC, 2004). A decreased risk for bladder cancer was reported for exposures to chloraminated water (Zierler et al., 1988; McGeehin et al., 1993) and ozonated water (Chevrier et al., 2004) in these studies. These studies provide some evidence to suggest that different mixtures of DBP that are produced by different disinfectant combinations might modify the risk of incurring different health effects.

UTILIZING TOXICOLOGICAL DATA ON COMPONENTS TO ASSESS THE SIMILAIRITY OF DBP MIXTURES

Toxicological data on individual components of DBP mixtures clearly can inform an evaluation of similarity among such mixtures. There are two parts of an individual component analysis that can inform similarity analyses: the array of toxicological effects associated with individual DBPs, and a consideration of toxicological properties that influence the identification of the components that are most important.

The first part is documenting the array of toxicological effects that are associated with a given DBP. This is illustrated in Table 3, where some of the principal adverse effects of different DBPs are identified. Adverse health effects associated with chlorination by-products include carcinogenicity, and reproductive and developmental toxicities. Liver, kidney, and central nervous system toxicities are reported in experimental animals treated with high doses of individual DBPs. Some DBPs have the potential of affecting thyroid function. However, while identification of these potential toxicities of the individual DBPs is useful, the magnitude of the component's contribution to the overall toxicity of the mixture also need to be considered. Thus, both qualitative and quantitative component information are essential to an analysis of whole mixture similarity.

Most of the chemicals listed in Table 3 are known DBPs; however, it is also important to consider the impact of postulated DBPs that have a high likelihood of occurrence, but for which survey data and/or health effects data are not available. While specific adverse health effects may be attributed to some DBPs, the toxicity testing of others has been limited to some measurement of mutagenic activity *in vitro*; thus, they cannot be assigned a quantitative estimate of risk. The halonitromethanes belong to the latter group. Table 3 includes haloquinones, organic *N*-chloramines, and alkaloidal nitrosamines as examples of the components for which neither occurrence nor health effects data exist. While widely varied potential harmful effects are associated with known DBPs, the magnitude of these effects cannot be estimated due to lack of necessary dose-response data. Nevertheless, evidence suggests that the nature of adverse health effects associated with DBP mixtures varies depending upon its composition.

The second part is consideration of toxicological properties that influence the identification of the components that are most important (Table 4). In addition to the question of primary effects, some secondary toxicological effects may be increased in importance as one considers other components of the mixture. It is important to ensure that the putative toxicological effect is relevant to humans. The other components reflect interactions between the concentration of the DBPs in the mixture and where human exposure doses "fall" on the dose-response curve. In the vast majority of circumstances, the human exposure dose will fall on that portion of the dose-response curve that is below the range of health effects observed in the experimental animal bioassay that was used to develop the dose response curve. Because typical rodent bioassays do not have the statistical power to detect effects using doses similar to those that humans experience through their uses of drinking water, the study doses utilized are significantly higher than typical human exposure doses. Consequently, dose-response modelers extrapolate their model results to this low dose and low response region. The extrapolation to low doses is influenced by knowledge of the carcinogen's mode of action (MOA): whether the chemical acts reversibly or irreversibly, and the implications of that MOA for humans, including sensitive individuals in the population. Finally, knowledge of the MOA of different mixture components provides a rational basis for considering interactions (e.g., synergism or antagonism) among components.

There are many DBPs in mixtures that have not been toxicologically characterized. An initial evaluation of how they might affect the toxicity of the mixture can be performed using approaches that were developed to deal with minor food and drug contaminants (Munro et al., 1996; Kroes et al., 2000). These methods serve to identify those products that are likely to be innocuous and to separate them from those that require chemical-specific toxicological data based upon resemblance to compounds for which data exist in the FDA databases on food additives and drugs. Quantitative

TABLE 3
Toxicologic Effects of Specific Disinfection Products

Mutagens	Mutagenic carcinogens	Nonmutagenic carcinogens	Reproductive toxicants	Thyroid effects	Developmental toxicants	Neurotox	Liver	Kidney
Established disinfection by-products								
MCA, MHAs	MX	Chloroform	DHAs	Chlorate	HAs	DCA	THMs	THMs
HANs	Bromate	TCA	HANs	HANs	HANs THMs	Chloral hydrate	HAAs	
Halonitromethanes	*N*-Dialkyl-nitrosamines	DHAs		Cyanogen halides	Chlorine dioxide			
Mono- and dihaloaldehydes		Chlorate						
Haloketones								
Probable disinfection by-products								
Organic *N*-chloramines	Haloquinones							
Organic *N*-bromamines								
Alkaloidal nitrosamines								

Note. MCA, mucochloric acid; MHAs, monohaloacetic acids; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone; HAAs, haloacetic acids; DHAs, dihaloacetic acids; DCA, dichloroacetate; THMs, trihalomethanes; HANs, haloacetonitriles; TCA, trichloroacetic acid.

TABLE 4
Critical Toxicological Properties of Individual Disinfectant By-Products (DBPs) That Will Influence the Judgment of Similarity Among DBP Mixtures

Nature of toxicological effects	Potency	Mode of action	Potential for interactions among components
Probability of affecting to different target tissues	Concentration	Reversible or irreversible	Modifications of metabolism
Change in tumor sites	Dose-response relationships	Genotoxic vs. nongenotoxic	Synergy
Interspecies extrapolation	Mode of action appropriate low dose extrapolation	Implications for sensitive populations	Antagonism

structure-activity relationships also aid such decisions (Woo et al., 2002; Bull et al., 2006).

Carcinogenesis

This section selectively reviews some of the changes in the toxicological properties of DBP mixtures that might be expected as a result of different conditions that occur among water supplies. In reading this summary, the reader is reminded that this discussion largely focuses on DBPs with substantial toxicological databases. A significant difficulty in addressing issues related to toxicological similarities among DBP mixtures using component data is that many DBPs can not be characterized toxicologically.

Disinfectant Used

Chlorine. Decisions related to dose-response evaluation have had to consider increasingly sophisticated datasets over time. These sophistications (e.g., physiologically based pharmacokinetic modeling and advances in the toxicological characterization of the MOA) provide an improved understanding of how changes in internal dose alters the molecular, cellular, and tissue-level processes underlying the observed toxic responses. Identifying key events that lead to the carcinogenic response frequently allows more accurate estimates of the doses required to produce the adverse effect at incidences too low to detect in experiments involving manageable numbers of animals. Since DBP mixtures are so complex, epidemiological studies cannot easily provide discriminators among chlorinated water supplies. Therefore, it is necessary to utilize the limited toxicological data that are available to determine whether some measures might be effective discriminators.

As indicated in Table 4, there are several toxicological characteristics of a DBP that govern the concentrations at which it will contribute to the health effects of the mixture. Classically, these variables fall into the phase of risk assessment categorized as dose-response characterization. As a first cut, DBPs may be grouped by whether their effects are thought to be cumulative and irreversible (e.g., mutagenic carcinogens) or, in cases where the effects are not cumulative, whether there is a dose below which any adverse response becomes improbable (a threshold dose).

The putative MOA of carcinogenic DBPs appear to differ widely and may be important factors in determining similarity of DBP mixtures with respect to cancer. A few of the carcinogenic chlorination by-products were shown to be mutagenic, including MX, HAN, and NDMA. The dose-response relationships assumed for chemicals that both are mutagenic and produce carcinogenic responses in rodent bioassays typically do not exhibit a threshold in the low-dose region (U.S. EPA, 2005). However, other DBPs (such as chloroform [U.S. EPA, 2001b]) were judged as unlikely to present a carcinogenic effect at the low doses associated with drinking-water exposures in the United States (U.S. EPA, 1998). Evidence that a mixture includes many DBPs with MOA for which a threshold may apply would suggest that it might be dissimilar from another mixture dominated by DBPs with nonthreshold MOA. Unfortunately, the MOA and key events associated with it cannot be determined for many DBPs from the available toxicological information.

The chlorination by-products that are recognized as being carcinogenic affect different target organs in different species. The different target organs of cancers produced by a DBP mixture can be used in assessing similarity, as it is likely to be dependent upon the DBPs that dominate the particular mixture. Such a determination may be used with caution, however, as epidemiological data identify target organs not observed in experimental testing of individual DBPs in rodents. Bladder cancer is the most consistent site associated with the use of chlorination in epidemiological studies, but bladder cancer in animals has yet to be demonstrated with any DBP studied (Morris et al., 1992; Mills et al., 1998). These are not necessarily contradictory findings as differences in target organs among species are not unusual. Further epidemiological studies evaluate humans who are exposed to DBPs through multiple exposure routes, whereas the animals in rodent bioassays are exposed only orally.

Colon carcinogens were identified in the limited number of compounds that were subjected to chronic study in experimental animals (i.e., BDCM and bromoform), but the potency of

those studied to date appears to be insufficient to produce the effect magnitudes reported at the low doses that are associated with epidemiological findings. In experimental animal studies of DBPs, liver, kidney, and thyroid cancer are routinely reported.

At present, the assessment of carcinogenic risk associated with different mixtures of DBPs largely relies on carcinogenicity data for a very small fraction of the DBPs in the mixture. As indicated earlier, QSAR studies suggest the possibility that some carcinogenic compounds are likely to be produced, but whose occurrence has not yet been confirmed (Bull et al., 2006).

The high concentrations of ClO_3^- that accumulate in hypochlorite solutions during shipping and storage have several implications for similarity of chlorinated DBP mixtures. The thyroid tumors observed in rats treated with ClO_3^- may result from this compound's ability to inhibit iodide uptake by the thyroid (i.e., this is likely the key event in the rodent MOA). As a number of other DBPs such as MX and HANs affect the thyroid, ClO_3^- may interact with these DBP or with other water contaminants, exacerbating this effect and enhancing the likelihood of thyroid tumor formation (Hooth et al., 1999). Interactions among thyroid active compounds also has implications for other adverse health outcomes not discussed in this article.

Chloramine. Chloramine treatment suppresses the formation of some halogenated by-products, especially THM. Mutagenic activity also is decreased (DeMarini et al., 1995). However, other by-products of potential health concern are formed with chloramination. These differences in the types of DBP formed are important variables in determining the similarity of different DBP mixtures.

The well-documented difference with chloramine is formation of nitrosamines when appropriate precursors are present. Most dialkylnitrosamines (e.g., NDMA, *N*-nitroso- *N*-diethylamine [NDEA]) are potent liver and esophageal carcinogens (Gold et al., 2007). Some longer chained nitrosamines (e.g., *N*-nitrosodibutylamine) are rat bladder carcinogens (Lijinksy, 1999; Ito et al., 1969). NDMA formed within the bladder *in vivo* was associated with increased incidences of human bladder cancer (Radomski et al., 1978; Singer et al., 1981).

NDMA is an important example of why more chemical-specific information is necessary to evaluate whether different DBP mixtures are similar. Significant levels of this potent carcinogen were produced in water that was treated with chloramine when dimethylamine precursors are present. Formation of nitrosamines with natural products in source waters has not been investigated. Tryptophan and its metabolite 3-methylindole are known to occur in natural waters and form nitrosamines (Bull et al., 2006). Since NDMA is 1000- to 10,000-fold more potent than THMs and HAAs, fairly low concentrations of NDMA or other nitrosamines might affect the projected toxicities of different DBP mixtures.

As pointed out earlier, dihaloacetonitriles tend to degrade in the presence of free chlorine. Therefore, higher concentrations might be expected if a given water source were to be disinfected with chloramine as opposed to chlorine. A recent NTP (2007b) study found dibromoacetonitrile to be carcinogenic. Thus, haloacetonitriles need to be considered in the assessing similarities among DBP mixtures.

A concern that has yet to be investigated fully is the likely preferential formation of halogenated quinones with chloramination versus chlorination (Heasley et al., 2004; Bull et al., 2006). Halogenated quinones are intermediates in the formation of the THMs and HAAs, as the aromatic ring is destroyed by excess free chlorine. Quinones are metabolic intermediates of a number of recognized carcinogens, such as benzene and polyaromatic hydrocarbons (Bolton et al., 2000). Structure–activity relationships suggest the presence of other by-products of chloramination that are substantially more potent than the regulated DBPs (Bull et al., 2006). If these compounds are found to occur even in the nanograms per liter range, they could be important determinants of similarity.

Chlorine dioxide. ClO_2^- is the by-product most closely associated with the use of ClO_2 as a disinfectant. The main adverse health effects associated with ClO_2^- treatment are anemia and methemoglobinemia. While some concern was expressed about the ability of ClO_2^- to interfere with thyroid function, this has not been a repeatable observation (Gill et al., 2000), nor is it likely based on the molecular size of this anion relative to ClO_3^- and perchlorate. Inhibition of the sodium iodide transporter is a function of the molecular size and symmetry of the interacting anion (Wolff, 1998). While ClO_2^- might exhibit such effects in isolation, it is unlikely to produce such effects at doses below those which produce oxidative damage to the red blood cell (Harrington et al., 1995a; Gill et al., 2000). ClO_2^- did not produce adverse developmental effects in rat pups at doses tolerated by the dam (Gill et al., 2000).

There are other unresolved concerns associated with the use of ClO_2 as a disinfectant. For example, it is not known whether it produces respiratory irritation from residual concentrations that approach those allowed in U.S. drinking waters (Daniel et al., 1990; Meggs et al., 1996; Olin et al., 2002) and whether it is a developmental toxicant (Aggazzotti et al., 2004; Orme et al., 1985; Taylor & Pfohl, 1985; Toth et al., 1990; Mobley et al., 1990; Carlton et al., 1991; Bull et al., 2008b). These are not addressed further given the focus on carcinogenicity.

Ozone. The principal by-product of recognized health concern that is produced with ozonation is bromate. Among recognized DBP, bromate is one of the more potent carcinogens (U.S. EPA, 2003). The current MCL was established at a level that could be measured reliably rather than at a specific health-based level. The MCL (10 μg/L) exceeds the estimated excess 10^{-4} lifetime cancer risk generally allowed in drinking water; thus, bromate levels near or above the MCL suggests dissimilarity from DBP mixtures containing little or no bromate.

As indicated earlier, O_3 produces some of the same brominated organic by-products that are formed with chlorine (e.g.,bromoform, dibromoacetic acid, dibromoacetonitrile), but at lower concentrations. On the other hand, O_3 produces higher levels of aldehydes than chlorine or chloramine (Koga et al., 1991); thus, aldehyde levels also need to be assessed in evaluating similarity among mixtures. The primary pathway and route of exposure for lower molecular weight aldehydes are likely to be inhalation while showering and other activities that result in volatilization within homes. The carcinogenic MOA for formaldehyde by the inhalation route is complex (Schlosser et al., 2003; Conolly et al. 2004).

Bromide

Bromine substitution is of likely importance because it is a "better leaving group," meaning that an organobromine compound is generally more reactive than the corresponding chlorinated analog. If not directly reactive, compounds with bromine substitutions are more readily metabolized to reactive intermediates that subsequently interact with DNA and other macromolecules. However, further substitution of bromine results in the formation of less stable reactive intermediates, causing them to react more readily with water, making it less likely that the active metabolite will reach a critical macromolecule.

From the perspective of carcinogenic responses to DBPs, one has to rely on the behavior of the classes for which cancer bioassays exist. Two changes occur with increased bromine substitution in the THM class, changes in the target organs in which cancer is induced and changes in potency. BDCM is the most potent carcinogen among the THM and affects multiple species and multiple target organs when administered using gavage in a corn oil vehicle (NTP, 1987), but, as mentioned earlier, not when administered in drinking water (NTP, 2006b). In corn oil, BDCM targets the liver and the kidney, but it produces much more robust effects in the kidney than chloroform. The induction of colon cancer in rat by BDCM distinguishes this THM from chloroform.

Bromine substitution in the THMs makes it a substrate of the glutathione *S*-transferase theta (GST-theta), which is much more effective in producing a mutagenic intermediate from brominated THMs than from chloroform (Pegram et al., 1997). The K_m and V_{max} values for activation of the brominated by-products are within the range of concentrations that are toxicologically relevant. The blood concentrations of chloroform needed to produce this mutagenic metabolite *in vivo* are lethal, so the MOA of chloroform does not involve this metabolite.

Some epidemiological data suggest that monobrominated DBP are more closely associated with adverse reproductive effects in humans than those that are completely chlorinated or more heavily substituted with bromine (Waller et al., 1998). These results have not been independently confirmed (Nieuwenhuijsen et al., 2000; Savitz et al., 2006), but they do support the concept that the relative degree to which by-products are brominated is important to the determination of similarity among DBP mixtures.

All of the dihaloacetates and trihaloacetates that have been tested for carcinogenicity produce liver tumors in mice. However, the MOA by which DCA and TCA induce liver tumors are distinct. While TCA acts as a classic peroxisome proliferator activated receptor alpha (PPARα) activator, DCA produces some substantial changes in insulin-related signaling processes in the liver that have physiological implications for the control of blood glucose at much lower systemic concentrations than those required for PPARα activation (Lingohr et al., 2001). Interactions between DCA and TCA demonstrated that the MOA of the two compounds are actually antagonistic to one another as tumor promoters (Bull et al., 2002, 2004).

All three dihaloacetates were evaluated for carcinogenic effects in mice. As indicated earlier, DCA is a liver carcinogen, but unlike TCA, it is capable of inducing liver cancer in multiple species (DeAngelo et al., 1996). Moreover, it has been convincingly shown that its ability to induce liver cancer is not dependent upon its weak activity as a peroxisome proliferator. Therefore, its carcinogenic effects are more likely to occur in humans, if the dose is sufficiently large (Bull, 2000). In lifetime bioassays, DBA was a liver carcinogen in mice at concentrations as low as 50 mg/L (NTP, 2007a). Unlike DCA, DBA did not produce liver cancer in rats, but it did produce malignant mesotheliomas and mononuclear cell leukemia. These latter target organs only offer some evidence of carcinogenicity (NTP, 2007a). Bromochloroacetic acid (BCA) was also shown to be carcinogenic in both rats and mice (NTP, 2008). In this case, there was clear evidence in both species, with rats developing malignant mesothelioma, large intestinal adenomas, and mammary-gland fibroadenomas and mice developing hepatic adenomas and carcinomas. All three dihaloacetates were evaluated in the shorter term studies. The dihaloacetates were progressively less potent liver carcinogens as bromine substitution increases (Bull et al., 2000). On the other hand, their activity as mutagens (Giller et al., 1997) rises with bromine substitution as does their ability to induce oxidative stress (Parrish et al., 1996). Thus, genotoxic mechanisms may contribute to the carcinogenic effects of brominated HAAs at low doses.

The only trihaloacetates that received study as carcinogens are TCA and bromodichloroacetic acid. TCA is problematic as a carcinogen in humans, in that it only produces cancer in the liver of mice; its effects have not been replicated in rats (DeAngelo et al., 1997). Unfortunately, bromodichloroacetic acid has only been studied in mice (Bull et al., 2000). Based on the mouse bioassay, it is somewhat less potent than TCA. On the other hand, it is clear that the mechanisms by which it produces liver cancer differ significantly from TCA. The carcinogenic effects of TCA depend upon its properties as a peroxisome proliferator. Bromodichloroacetic acid is not effective in activating this mechanism (Kato-Weinstein et al., 2001). It is considered unlikely that compounds that act through this mechanism would be carcinogenic in humans because activation of the PPARα does not induce the same pleiotropic responses in human or other primate livers as seen in rodents.

Bromodichloroacetic acid, on the other hand, is a multiorgan carcinogen in mice. It produces lymphoma and lung tumors in mice as well as liver tumors. Moreover, it appears that its carcinogenic effects in the liver might depend upon the fact that DCA is one of its metabolites (Merdink et al., 2001).

All classes of halogenated DBPs follow the relative proportion of bromine substitution that is observed with the THM and HAA classes. Thus, DBP mixtures from water supplies with significant amounts of bromide in the water need to be carefully evaluated for compositional similarity (dissimilarity) from those from water supplies where bromide is low. The question of whether the relative potency of DBP as colon carcinogens relates to the degree of bromine substitution is important and needs to be a major focus of future research on the toxicology and similarity of DBP mixtures.

The data that are available on the two major classes of DBP whose carcinogenic activities that have been well characterized suggest that the monobrominated forms, BCA and BDCM, are more consistently carcinogenic. They produce cancer in both mice and rats, produce multiple tumor types, and/or are more potent as carcinogens than their fully brominated congeners.

pH

pH levels are important in the assessment of similarity among DBP mixtures. As the reaction mixture into which chlorine is introduced tends toward more acidic pH, there are two variables that need to be considered somewhat independently. First, mutagenic activity in *Salmonella* tester strains increases. Second, the relative yield of TTHMs will be less compared to trihaloacetic acids and aldehydes and ketones with trihalogenated carbons. Within these classes, the MOA by which DBPs produce adverse effects appear to be diverse. Some of the halofuranones, haloketones, and haloaldehydes, whose formation is favored at acid pH, exert genotoxic effects (Robinson et al., 1989) that contrast with the nongenotoxic carcinogens, DCA and chloroform. Therefore, genotoxic mechanisms are favored at more acidic pH. The differences in mode of action affect how effects of individual chemicals at low doses are considered in risk assessment. In addition, differing MOAs in a mixture imply different types of interactions among chemicals because it suggests the possibility of synergisms.

The most mutagenic by-products in bacterial test systems produced by chlorination are MX and its analogs. The most sensitive target organ for the carcinogenic effects of MX is the thyroid. As a mutagen, its effects on this organ would be considered linear with dose. The formation and stability of other mutagens formed with chlorination is also enhanced at low pH, although target organs have not been defined. Some showed capability of initiating tumors in the mouse skin and inducing lung adenomas in strain A/J mice (Robinson et al., 1989; Bull & Robinson, 1985). Therefore, it appears at least some of the mutagens produced at acid pH are also tumor initiators.

Thyroid cancer might be initiated by MX, but clearly ClO_3^- could act as a promoter of thyroid tumors. Similarly, nitrate that might be incidentally present (i.e., it is not a DBP, but is a common contaminant of drinking-water sources) in the same water could act to promote thyroid tumors. This sets up a classic case for synergism. DBP mixtures that have either of these two anions present at concentrations that affect the thyroid are considered different from DBP mixtures that contain substantially lower concentrations, as these scenarios indicate differences in their potential cancer risks.

Temperature

Based on current knowledge, the effect of temperature is to modify the amounts of disinfection by-products that are produced in any given water supply. Other variables appear more likely to affect the nature of the by-products that would be formed. The MOA of the mixture may change with these other variables (e.g., pH, ammonia, bromide), but there is no information to document differential rates of formation among DBP classes as temperatures change. Temperature also affects the rate at which less stable by-products are degraded. DBPs whose degradation rates are temperature sensitive may be important contributors to differences among DBP mixtures. The possibility that DBP mixtures might vary by differences in temperature during treatment, distribution, and storage has not been systematically studied.

Association of Mutagenic Activity With Adverse Health Outcomes

A study associated mutagenic activity in chlorinated drinking water with increased cancer risk. Koivusalo et al. (1994) found a relative risk of 1.2 for bladder cancer and 1.2–1.4 for kidney cancer in Finland. The odds ratios for bladder cancer are no greater than those observed in other water supplies when chlorinated supplies are simply compared to nonchlorinated water; thus, mutagenic activity appears not to be any more of a discriminator than other parameters. The increased risk of kidney cancer has not frequently been associated with chlorinated drinking water. Further research is needed to examine this latter association.

Despite this epidemiological study, it is difficult to relate a higher level of mutagenic activity in drinking water to actual risk. A serious question arises about the adequacy of measures of mutagenicity in drinking-water extracts for providing insight into the character of a particular mixture of DBPs and consequently its similarity to other DBP mixtures. The most serious problem is the lack of specificity and probable sensitivity to substantial shifts in the composition of a DBP mixture. For example, one can easily envision a circumstance where some mutagens increase while others decrease with a change in conditions. The general dependence upon the standard plate assay using a single *Salmonella* tester strain in the absence of metabolic activation as the only measure of such effects in surveys provides little power for detecting shifts in composition. Variations in the *Salmonella* mutagenicity assay, such as the use of other means of metabolic activation and other methods of

introducing the chemicals, might provide more specificity to the characterization of a particular water supply and serve as a discrimator among DBP mixtures.

Several authors in the late 1980s published extensive analyses of the relationship between mutagenic potency in simple tests and carcinogenicity (McCann et al., 1988; Parodi et al., 1983; Ashby & Tennant, 1988). Findings were uniform that such correlations are weak and certainly not to be depended upon as indicators of health effects from complex mixtures. Further, the *Salmonella*/microsome system cannot detect most types of interactions that are involved in generating synergistic effects because of the simpler physiology and overly simplistic nature of the metabolic activation systems that are used compared to the *in vivo* situation in humans.

SUMMARY AND CONCLUSIONS

This review of the literature clearly indicates that varying water conditions and chemical disinfectants can have substantial effects on the type of by-products that are formed. Data show that a number of other factors influence the formation and occurrence of certain by-products. Some of the differences can be inferred from the chemical conditions in the water being treated and/or by examining relationships seen in forms of DBPs that are commonly measured in drinking water. Consequently, some use may be made of existing data to determine whether mixtures of chlorination by-products differ. Confidence in such determinations would be strengthened if the occurrence of less abundant DBPs was more frequently reported than it is at present. Most important, the toxicological database for individual DBP and classes of DBP needs to be more robust. Finally, the confidence in assessing DBP mixtures would be significantly improved if the epidemiological and toxicological data sets approached concordance.

Table 5 identifies some parameters that might be derived from available data to make judgments about the similarity of DBP mixtures obtained from different geographic locations or during different seasons of the year. Confidence in judgments of similarity based on these currently available data may be limited. Some of these are developed more formally in Bull et al. (2009a). The table also suggests other parameters that could be developed from current measurements of DBP. A more difficult task is discerning the nature and contribution of uncharacterized DBPs to dissimilarities among mixtures. It is suggested elsewhere that progress in this area may be aided by the use of chemical and toxicological structure activity relationships (Bull et al., 2006) to identify new DBPs and their likely toxicology. Finally, the table identifies a limited research agenda that would increase the credibility of judgments about the similarity of DBP mixtures.

TABLE 5

Parameters That Would be Useful in Characterizing Differences in Mixtures of Chlorination By-Products

Derived parameters

1. The fraction of the total organic halogen (TOX) accounted for by measured chlorination by-products
2. The relative amounts of known chloro-, bromochloro-, and bromo-by-products can be extrapolated to the unknown portion of the TOX
3. The dihaloacetate/trihaloacetate ratio
4. The trihaloacetate/trihalomethane ratio

New parameters and data reporting conventions that should be developed

1. Separate reporting of the TOBr and TOCl
2. Formation potential for nitrosamines
3. Separate reporting of disinfectant by-products (DBPs) with differing numbers of halogens on a single carbon (e.g., the dihaloacetic acids and the trihaloacetic acids)
4. Analytical methods for critical compounds not currently monitored
5. Markers for site-specific variation in natural organic matter (NOM) based on yield ratios with less routinely measured minor DBPs and an analytical use of mutagenicity assays

Research needs

1. Assessment of how qualitative differences in NOM influence formation of nonregulated classes of DBPs (see suggested measures of ratios between major and minor DBPs)
2. Surveys that validate the use of surrogate measures for occurrence of nonregulated classes of DBPs—include “diagnostic” approaches using mutagenicity testing
3. Studies of how conventional water treatment processes alter the precursors for different classes of nonregulated DBPs

TOBr = total organic bromine; TOCl = total organic chlorine.

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